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Public Health Reports

Treasury Department, United States Marine-Hospital Service. Published in accordance with act of Congress approved February 15, 1893.

VOL. XV. WASHINGTON, D. C., MARCH 2, 1900. NO. 9.

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UNITED STATES.

EXPERIMENTAL PNEUMONIC PLAGUE.

[By Dr. Batzaroff—Translated by P. A. Surg. H. D. Geddings from the Annals of the Pasteur Institute, May, 1899.]

If we review the history of the epidemics of plague which have prevailed for centuries in different parts of the Old World, and to which we have given the name of plague, we see that respiratory troubles have been always mentioned as among the symptoms which frequently accompany this disease. Moreover in some epidemics, and especially in those which have been justly regarded as the most terrible, we find the lungs almost exclusively involved.

The epidemic which decimated Europe and Asia under Marcus Aurelius and that of the years 255–265 B. C., of which St. Cyprien has given us a faithful description, the great plague of Justinian in 542 and the terrible "black death" or "black plague" of the fourteenth century which carried off a quarter of the population of Europe must be included in this category.

In fact in all these epidemics the bubonic cases were relatively rare or were entirely wanting, which led certain historians to believe that they were dealing with a disease different from ordinary plague (they used to speak of malignant epidemic fever), for the bubo was always considered most characteristic of the plague and even up to recent times they maintained "that there was no plague without buboes," hence the current name of "bubonic plague."

Without being able to prove it in any very definite manner, it is probable, however, that the great epidemics which we have just mentioned, were epidemics of plague, but of pulmonic plague. In fact the existence of a pneumonic form of plague could not very long escape careful observers. At first they used to speak of "pulmonary complications" which were produced during the course of a plague infection, and which were generally attributed to a secondary infection; but, dating from the little epidemic of Wetljanka in 1878–1879 the name "epidemic pneumonia" came into current use, but at the time we could go no further, for in spite of all our assumptions that the disease might be entirely confined to the lungs, further proof of it was impossible at that period. It was only after the discovery of the specific agent of plague by Yersin and Kitasato that we could furnish this proof.

In December, 1896, Childe, professor of pathological anatomy at Bombay, basing his conclusions on the examination of the sputum during the disease and upon the necropsic findings in a certain number of plague cadavers, demonstrated the existence of a primary pneumonic plague.

Two members of the Russian commission to Bombay, Wissokowitz and Zabolotny, observed in 1897 several cases of plague pneumonia, and were able to reproduce the disease experimentally in the monkey. Since that time the fact established by Childe has been confirmed by all those who have had an opportunity to study the plague in patients during the late epidemics in Asia.

At the present time it is actually established that coincidently with the bubonic form of the disease, which is but a mild form of the plague among human beings, there exists "a plague without buboes," which takes the clinical course of a pneumonia. Very frequent in some epidemics of plague, less so in others, pneumonic plague is one of the most dreadful forms of the disease. Hence the indication to seek experimentally the track by which the plague virus enters the economy, what is the course of the disease, and what are the prophylactic measures which can be taken to prevent or cure. This is the subject of this work.

Primary pneumonic plague.

In animals, as in man, we meet two different forms of pneumonia—that is to say, primary pneumonic plague and secondary pneumonic plague. Let us commence with the consideration of the first.

As we have just said, immediately after the proof of the existence of pneumonic plague among men by Childe, Wyssokowitz and Zabolotny tried successfully to produce the disease in monkeys by introducing the plague bacillus into the trachea of the animal by means of a sound, under chloroform anæsthesia. This procedure, though ingenious, is very inconvenient above all when it deals with small animals as rats and mice. Hence they endeavored to find another more closely approaching the mode of natural infection.

(*a*) If we deposit on the nasal mucous membrane of an animal, without excoriating it, a little pest virus, we give that animal a pneumonic plague with great certainty. In the guinea pig, which is the animal we preferably use in our experiments, as in the rabbit and the monkey, this little operation may be very easily performed by means of a fine glass rod, previously sterilized, whose extremity is wrapped in cotton to avoid all chance of wounds. But in rats and mice the operation is more difficult to perform on account of the narrowness of their nasal passages; hence we have usually been satisfied with spreading a little of the virus on their nostrils. In these little rodents the infection does not as invariably ensue as in monkeys, rabbits, and guinea pigs, but if we operate on 10 mice we will always have at least 5 or 6 which will contract the disease and die of it.

The virus introduced into the nostrils consists of a small quantity of an agar culture of the B. pestis, or bacilli in the spleen of a plague-stricken animal. Bouillon cultures are not suitable for this species of inoculation, for they are soon expelled by the sneezing of the animal or the slight secretion which follows the introduction of the virus.

When the virus is virulent the disease always presents a regular course. During the first twelve hours we often find a lowering of the temperature of the body, which falls to 37° C., and sometimes lower. This is probably a reflex effect of the irritation of the sensory nerves of the mucous membrane. Then the temperature slowly ascends and remains within normal limits until about thirty hours after the inoculation. During this time the animal eats and appears well, so that nothing in his general condition shows the gravity of the disease with which he is threatened. Bacteriological examination alone at this time permits of a diagnosis, for a trace of the nasal mucus, taken from the inoculated side, and examined under the microscope, shows a quantity of plague bacilli in rapid proliferation, as evidenced by the small size of the elements which make up the culture.

But the picture soon changes; the temperature mounts rapidly to 40.5° to 41° C., and in some cases to 42° C., and is maintained at this figure; the animal becomes sick, ceases to eat, his hair becomes roughened up, his respiration quickened. This condition of illness goes on increasing, failure is rapid, the respiration becomes more and more rapid and painful, and is at the same time strident and puffing. The animal commences to cough and to throw off a small quantity of foamy reddish fluid. The nasal secretion increases, and the liquid of which it is formed dries in small yellowish crusts around the nostrils. Through the naso-lachrymal duct the inflammation extends to the conjunctiva, and the conjunctivitis, which follows, attacks first the eye corresponding

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[a] This fact was mentioned by Professor Roux in a preliminary note in 1898.

to the side of inoculation. The febrile condition lasts generally twenty-four to thirty-six hours; then the temperature commences to fall and the guinea pig dies at the end of the third or the commencement of the fourth day, after having presented a tremendous dyspnœa and hypothermia. If the disease is prolonged there is often produced a muscular paralysis of the stomach and intestines; the abdomen is distended, swollen, and the displacement of the diaphragm toward the thoracic cavity, consequent upon the enormous dilatation of the stomach, also contributes to the suffocation.

On necropsy we find the lymphatic glands increased in size and forming secondary buboes; occasionally we see true primary buboes formed usually by the deep lymphatics of the neck. Once only have I seen an axillary bubo on the side of inoculation; the tumor which had the form and color of a bean was bloody, of a soft consistency, and contained a great number of specific organisms.

When we open the thoracic cavity we note that the lungs which are greatly dilated do not retract. In a certain number of cases they are much congested, in other cases the congestion is less marked, and here we see the lesions best. These are, first of all, small miliary hemorrhages on the visceral pleura, then pulmonary infarcts more or less extensive, multiple areas of infiltration, which are sometimes limited and sometimes diffused, or separated by normal lung tissue; sometimes, on the contrary, the contiguous areas unite and may occupy an entire lobe. In cases further advanced we may see areas of true hepatization. All these pulmonary lesions are more pronounced on the side of inoculation than on the opposite side, when we have made a unilateral inoculation.

Both lungs are œdematous. When we cut the diseased parts a bloody liquid containing plague bacilli even in pure culture exudes, though there may be an association with other organisms. In the pleural cavity and in the pericardium we often find a small quantity of exudate, clear, straw colored, or opalescent and of the color of the juice of gooseberries, in which microscopic and cultural examination will show the presence of the plague organism. The heart itself is greatly dilated, especially the right heart. The epicardium sometimes shows small multiple hemorrhages. These hemorrhages can often be recognized on the peritoneal surface. The abdominal cavity contains a little exudate. The spleen is large, of a deep red color, of medium consistency, and its granular surface is covered with a great number of small white points. This "punctate" spleen which is very characteristic of the disease contains an innumerable quantity of plague bacilli. The stomach, as we have before said, is much dilated. In the cases where the muscular layer has been paralyzed it presents numerous small hemorrhages, situated especially on the great curvature. These hemorrhages which involve the gastric mucous membrane, appear from the serous side as small, regular, round, blackish points, surrounded by an area of pale gray. The paralysis extends to the duodenum and the adjacent parts of the small intestine. The liver is large and granular, the cystic duct obstructed, and the gall bladder much dilated. The kidneys and the suprarenal capsules are congested and often streaked and marked with hemorrhagic points.

The heart blood, taken after death, and planted gives a pure culture of plague bacillus.

Microscopic examination of sections shows a general congestion of the pulmonary tissue, a tumefaction of the bronchial mucous membrane, and a catarrhal exudate. Numerous areas of broncho-pneumonia exist; in these areas the alveoli are found filled with an exudate consisting of desquamated cells of the alveolar walls, mono-nucleated leucocytes, red blood cells and numerous plague bacilli.

The sum of this brief résumé of the clinical course of the disease is that the pneumonia which follows the introduction of plague virus into the nose of a susceptible animal is a broncho-pneumonia, but that the virus passes out of the lungs and generalizes itself, and that the animal succumbs to a septicæmia accompanied by an alveolar œdema of the lungs.

Contagiousness of pneumonic plague.

The mortality of this form of pneumonia among animals is 100 per cent. Is this pneumonia contagious; can it be transmitted from animal to animal by contact? The experiments made in this line permit us to give an affirmative reply. They have shown, first, that all the secretions and excretions of the sick animal—above all the liquid which flows from the nose, that secreted by the conjunctiva when that membrane is affected, the matter thrown off from the mouth during an access of coughing—when conveyed into the nose of a healthy animal give to it the plague. But the disease is permissible also by propinquity. On several occasions we have placed animals in the cages which held those inoculated through the nasal passages. In a majority of instances these animals have contracted the plague, and at the necropsy the absence of all buboes and existence of the pulmonary lesions proved in a certain manner that it was through the respiratory tract that the infection had been produced. Contagion is easily produced if the animals are fed on carrots or beets, because healthy guinea pigs may easily soil their noses in nibbling at food contaminated by a sick animal.

The quantity of virus which suffices to give pneumonia to a susceptible animal is very small, and it is not even necessary that it should be virulent in order that the infection may occur. In our old culture from various sources we have chosen one which had lost all virulence. In the course of our preliminary researches we have found that the plague bacillus did not always preserve its characteristics; in other words, that there exist varieties of the organism. In general we may say that it is an error to consider the organism of human bubonic plague as a very weak one, for if there are some species of it which very quickly become attenuated, and die outside of the human body in a relatively short time, there are others which under the same conditions preserve their vitality for a long time, and grow on artificial media for months and even for years without any noticeable loss of virulence. We have seen that some of our cultures kept in the laboratory without any special precautions, exposed to light for three and a half months, killed animals by hypodermatic injection with only a slight delay, and replanted three or four times showed themselves as virulent as they were in the beginning. The attenuation of a plague culture, then, is not always easy to arrive at, and requires a combination of circumstances and considerable time.

Several months ago we received three new specimens, all plague cultures, all authentic in their origin and morphological characteristics, yet which had lost their virulence completely. They were purely saprophytic for the animal economy, for inoculated in large doses into mice and rats they made them scarcely sick, and guinea pigs did not suffer any inconvenience.

We introduced into the nose of a guinea pig one of these cultures of human origin, and grown for twenty-four hours on agar. The first passage was lethal, the incubation was eight days, and on the eighth day the animal succumbed with a typical pneumonia. With the spleen of this first guinea pig we inoculated a second, then a third, and so on in series; at each passage the duration of the disease diminished, the period of incubation became shorter so that at the end of these successive passages the animals died as quickly as those inoculated with our ordinary virulent virus.

But the fact which we have just mentioned seems to us important, especially from its bearing on the epidemiology of plague. In fact, Yersin, after his notable discovery of the plague bacillus, in examining the soil of a native dwelling place, found at the depth of 4 to 5 centimeters a microbe similar to that of plague, but nonvirulent for laboratory animals. It was probably a plague bacillus attenuated. After what we have just proved for our bacillus, which was quasisaprophytic, may we not admit that this attenuated virus might at any moment, under certain favorable conditions, penetrate through the nasal passages into a susceptible animal, such as a rat or a mouse, and after several such successive passages, regain all its virulence.

Certainly this is not a mere hypothesis; it appears very probable to us and merits close investigation by all those who have opportunity to study plague on the spot; in this connection it would be very interesting to know what are the lesions observed in animals dead of spontaneous plague, and if they always present the primary bubo.

Moreover we have been enabled to prove that even in a dry condition the plague virus remains for a long time living and virulent. For this, organs taken from animals dead of plague, as well as plague cultures mixed with infusorial earth, were submitted to drying in vacuo at room temperature. From time to time we took out a little of the material, broke it up in a sterilized glass, and the powder thus obtained was introduced in small quantity into the nose of an animal. The results of these experiments, which are shown in the adjoined table, demonstrate that the plague virus bears very well a prolonged drying, when it is in albuminous media, such as spleen pulp or that of any other organ. Under these conditions it is attenuated very slowly, so that in the first three or four weeks the attenuation is insignificant. When, on the contrary, it is not so protected, as is the case with the culture dried in infusorial earth, its virulence is rapidly lowered in the first few days, and at the end of three weeks nasal inoculation no longer produces any morbid effect.

Plague-pneumonia produced by inoculation (nasal) of dry virus.

Splenic pulp.		Splenic pulp.		Cultures dried on infusorial earth.	
Duration of drying.	Duration of disease.	Duration of drying.	Duration of disease.	Duration of drying.	Duration of disease.
Days.	*Days.*	*Days.*	*Days.*	*Days.*	*Days.*
5	4½	26	4½	2	7
8	4	30	6½	5	6½
12	4½	33	6½	7	(*a*)
14	5	38	6½	19	12
20	4½	44	(*b*)	37	(*b*)

a Resisted. *b* Resists.

In the presence of these experimental facts, it seems to us that we have every right to maintain that the sputum of plague patients, whether pneumonic or otherwise, the bodies of animals dead of plague, and every other organic albuminous substance containing the virus of plague, whether in a fresh state or whether dried, play an important rôle in the propagation of human plague in general and the pneumonic form in particular.

The nasal mucous membrane is not the only one which lends itself to the penetration of the plague virus into the economy of animals. All the accessible mucous membranes enjoy this property to a greater or less extent. Without further insisting on this point, very interesting inasmuch as it concerns the pathogenesis of the disease, we will limit ourselves here to recording the fact "that in point of view of facility of infection the various mucous membranes may be classed in the following order: Nasal, conjunctival, buccal, intestinal, and last, vaginal." In fact, the disease produced by the deposit of virus on the vaginal mucous membrane usually has a subacute character. We sometimes meet animals which do not contract plague by this route. It is very probable that in these cases the vaginal mucus does not suit the plague bacillus; in the liquid which flows we sometimes find plague organisms mixed with many other species.

To every mucous membrane there is a corresponding form of the disease and a special category of symptoms which would be too long to enumerate here. Let us simply note that in the case of intestinal plague, caused by the injection of substances containing the plague bacilli, the mesenteric glands, which represent the primary buboes, may attain the size of hazelnuts. In the case of rectal infection we observe large inguinal buboes on both sides, and a swelling of the lymphatic glands of the mesentery. Finally, when the vagina is the point of departure of the disease we find quite a chain of glands which ascends along the spinal column. It is very evident that inoculation with plague is an excellent method for studying the distribution and anastomoses of the various lymphatic vessels, as well as their relation with the glands of the affected region.

II.

Secondary pneumonic plague—Pathology.

The second form of pneumonic plague which is to be mentioned is not a specific disease like the first; it is secondary pneumonic plague, a complication which we meet frequently in animals stricken with bubonic plague, but which may be developed quite as well in the course of any plague infection, regardless of the avenue of entrance of the infection, provided that the economy opposes a certain resistance to the invading bacillus. This resistance is sometimes natural, sometimes acquired in consequence of the introduction into the body of some vaccinating substance, such as plague organisms killed by heat, or antipest serum, in insufficient quantity to render the animal quite refractory to the disease, but sufficient to stimulate the economy to defense in the struggle which is waged between it and the morbific agent, and to ward off in this way a fatal issue.

The special characteristics of secondary plague pneumonia is the formation in the lungs, in greater or less number, of lesions which present to the eye such a resemblance to true tubercles, that seeing them for the first time we may think that we have to deal with a tuberculous affection. These pseudo-tubercles, for we will see that they have nothing in common with true tubercle beyond their external appearance, have their seat of election in the superficial part of the lungs. In the beginning of their formation we see appearing under the visceral pleura, numerous whitish points, of variable size surrounded by a reddish zone of reaction, which increase in volume. The pseudo-tubercles already formed are shown under the form of round or slightly elongated bodies, white or dirty gray in color, whose convex surface showing below the pleura, gives the lung a granular appearance.

These formations are sometimes very numerous, scattered over the entire pulmonary surface, and then they are small like grains of millet or lentils; sometimes their number is limited, but as if in compensation for this they may attain the size of a pea. In quite exceptional cases we have observed true tumors as large as hazelnuts. Sometimes we see that by the aggregation of several tubercles a part of the lung undergoes a transformation into compact tissue, completely deprived of air, which sinks to the bottom in water. We would say that the lung was in a state of grey hepatization. To the touch the pseudo-tubercles give the impression of hard bodies, and when we press them between forceps, there exudes from the cut surfaces a turbid grayish fluid, containing besides enormous numbers of plague microbes, a certain number of cells, the greater part of which are mononucleated leucocytes.

The lungs containing pseudo-tubercles are in general enlarged and highly congested. On microscopic sections, stained with thionin, we see that the vessels are very dilated and filled with blood, but there is a lack of perivascular reaction. Small hemorrhages soak the pulmonary tissue in different localities. The lung structure is in its entirety seriously altered, so that in certain foci it is impossible to recognize it. Where it is still recognizable, we note an enormous thickening of the septa which limit the alveolar walls and the pulmonary lobules, due to a marked leucocytic infiltration.

Between the leucocytes we see an enormous number of plague bacilli, and sometimes, though not often, we even find them in the interior of the leucocytes. The elements which compose this infiltration are in the beginning quite well preserved, but in a more advanced stage these elements are destroyed and we only see large tracts of amorphous masses, composed of cellular detritus. The greater part of the alveoli, equally dilated, are filled with an exudate formed of desquamated epithelial cells, leucocytes, and red globules; in the midst of the cells we find a greater or less number of bacilli. The elements which go to make up this exudate are sometimes well marked, in others they are deeply altered and in process of destruction. Then the alveolus is seen to be filled with a granular amorphous substance, streaked here and there with small colored areas, the residue of destroyed nuclei, of some elements which we recognize by their nuclei in spite of their great alteration and great numbers of microbes; they are veritable necrotic areas.

In the areas corresponding to the pseudo-tubercles, of which we have just given a lengthy microscopic description, the pulmonary structure is irrecognizable. Situated in the periphery of the lung under the pleura, these tubercles are formed of an enormous aggregation of round cells, especially of leucocytes, the greater part of which present great alterations in their plasma and nuclei, some remains of a stroma of connective tissue and almost a pure culture of the plague bacillus; the whole is surrounded by a zone of very great vascular reaction and small disseminated hemorrhages around the periphery of the pseudo-tubercles. According to this microscopic picture we see a total lack of the elements which go to make up true tubercles, viz, the giant and epithelioid cells.

As produced experimentally.

This form of experimental secondary plague-pneumonia does not resemble any other form of pneumonia which we are likely to encounter. We can now only say with certainty that it is not of embolic origin, but that its point of departure is in the lymphatic system of the lung.

The secondary plague-pneumonia, such as we have just described, is an evidence in animals of an insufficient defense against microbial invasion. To provoke it experimentally we have two methods at our disposal: (1) To employ an attenuated virus; (2) to introduce into the animal economy a small quantity of a vaccinal substance. In fact, when the virus is very active and death supervenes in three or four days, the lungs only present a simple congestion; the principal lesions are found in the spleen, which is large and covered with white points. If, on the contrary, we use a virus which kills the animal in seven to nine days, we may often obtain, though it is not the rule, the formation of typical pseudo-tubercles, but in these cases the other lesions are also present but are not so pronounced. The small points in the spleen are transformed into small miliary abscesses, and we see similar abscesses on the surface of the liver and kidneys.

The second method is much more certain and convenient, that is, the injection of a vaccinal substance. In making these experiments, and by employing more or less powerful doses of the prophylactic substances, we succeeded easily in producing the whole gamut of lesions, and arrived at the singular conclusion that in proportion as the lesions of other organs diminished those of the lungs increased, so that at a given moment, when the animal was at a point of absolute immunity, the pulmonary lesions were the only ones to be discovered at the necropsy; all the other organs, notably the spleen and the liver, preserved their normal appearances, and the plague virus only existed in the lungs and the pseudo-tubercles.

The property of producing a pneumonia with pseudo-tubercles in the guinea pig does not belong exclusively to the plague bacillus. The same lesions have been noted in certain septicæmic diseases, among others, in those due to the microbe of hog cholera, described by DeSchweinitz and Theobald Smith. It is one fact the more which pleads for a common origin, already so striking, of the plague bacillus and other hemorrhagic septicæmias.

But the experiments related above present in our judgment even a more general interest from the point of view of immunity. It appears in fact that each organ has an immunity proper to it, and that general immunity is only acquired when all the organs are immunized. In this respect all the organs do not deport themselves in the same manner, for the result of our experiments shows that the spleen and the liver, for example, are early vaccinated against the plague, while the lung only comes in at the last moment. What is the cause of this inferiority of the lung? Does it depend on the question of aëration which facilitates the growth of the microbe? Is it due to the poverty of these organs in phagocytic elements, or are there several factors which come into play? It is evident that this is a question which necessitates special and deep study for its solution. Let us for the present be content with noting the fact without seeking to draw any conclusions.

III.

Immunization with antipest serum.

After having produced in the guinea pig the two forms of plague pneumonia, we tried also the prevention and cure of primary pneumonic plague by means of antipest serum, but before reporting the results of these experiments it appears to us useful to say a few words on the subject of the preparation of the serum itself. The sero-therapy of plague was inaugurated in 1895, when, under Roux' direction, Yersin, Chalmette and Borrel undertook the immunization of small laboratory animals by first injecting them with cultures killed by heat, then with live germs. A horse which had received injections composed of living and virulent cultures furnished a serum which immunized mice in a dose of 1/10 c. c., twelve hours before the injection of the virus; in the dose of 1.5 c. c. it cured mice inoculated twelve hours before with a platinum loopful of plague virus. This first serum used by Yersin in 1896 in the treatment of certain cases of bubonic plague in Canton and Amoy gave excellent results.

In spite of its efficacy this method of preparation of antipest serum by means of living cultures presents many dangers. Therefore it has been endeavored to immunize large animals in a less dangerous manner by injecting them either with cultures killed by heat or by means of substances extracted from the bodies of the microbes; these methods do not furnish a sufficiently active serum.

Departing from the idea that a serum curative for the plague should be essentially antitoxic, some people have endeavored to imitate the current process for the manufacture of antidiphtheritic and autitetanic serums, by employing for the immunization of animals not the microbial bodies, but the soluble products elaborated by the microbes when cultivated in liquid media. Now the efforts made in this direction have not given a satisfactory result, perhaps because one has not yet succeeded in preparing outside of the body a sufficiently strong toxin. Roux was the first to prove the existence of soluble toxic products in the culture media of plague. By using a virus the virulence of which had been much increased by successive passages through animals in small collodion placed in the peritoneal cavity, Roux was enabled to obtain a virus which killed mice in a dose of 1/70 c. c. in twelve hours; but these same toxins showed little activity for rabbits and especially for guinea pigs. Later on Markl pretends to have prepared a toxin fatal to mice in doses of 1/200 c. c. and which kills a guinea pig in eight days in the dose of 0.5–5 c. c., but he gives none of the procedure which he has employed in his experiments; but even Markl has demonstrated that with a toxin as active as his we can not confer on an animal an immunity active, absolute, and durable against a virulent inoculation, and that we can not obtain a serum which responds to all requirements.

In the presence of all these facts we must ask if we find in bouillon culture medium the true plague poison, that is to say, the same poison which the microbe secretes in the animal economy. It is certain that this poison is not formed at a low temperature, and that once formed it is unstable, for it is rapidly destroyed under the influence of heat, oxygen, air, and light.

The combined method upon which Markl depends for the production of an efficient antipest serum, and which consists in the simultaneous employment of soluble toxins and dead microbial bodies is not new. It has long ago been tried at the Pasteur Institute, but has been proved to possess no advantage in the quality of the serum produced. Immunization with living virulent bacteria has up to the present time given the incontestably best results. It should be carried on for a long time and with prudence, for if we act rashly we run the risk of losing our animals; moreover, all horses immunized under the same conditions do not give an equally good serum. We must, therefore, treat several animals at the same time in order to select the best. The serum thus obtained, as Roux has demonstrated, is at the same time both curative and preventive. One-twentieth c. c. of the serum thus obtained preserves mice against a virus which kills in three days, and 1/4 c. c. cured when injected twelve hours after a virulent inoculation. If we use a virus which kills in thirty-six hours, the doses of serum necessary to preserve and cure mice are $\frac{1}{10}$ and $\frac{1}{2}$ c. c., respectively. The guinea pig is a very susceptible animal to plague, and it is difficult to render it immune to the disease; this is a fact recognized among all experimenters. Nevertheless, with a good serum we can immunize this animal against the disease and arrest it when it has already commenced. Thus, 1 c. c. of serum mixed with a surely fatal dose of plague culture, renders the latter innocuous to the guinea pig. The guinea pig which has received 1–2 c. c. of the serum twelve hours before the virulent inoculation does not acquire the disease. We may even cure the guinea pig by injecting 3–5 c. c. of the serum twenty-four hours after the subcutaneous inoculation of the virus. Finally, 3 c. c. of serum, injected under the skin of a guinea pig, protects it against the intraperitoneal inoculation of a dose of plague virus which kills the control in forty-eight hours. All these experiments have been made with animals weighing from 500 to 700 grams.

It is with this serum that we have tried the sero-therapy of the primary plague-pneumonia of the guinea pig. Numerous experiments made to this end have shown that 1 c. c. of the serum injected twelve hours before the nasal infection is sufficient to preserve the animal when the virus was derived from agar culture, but if we use splenic pulp for the inoculation, the dose of the serum necessary to prevent the disease must be at least 2 c. c.

The efforts to cure plague-pneumonia by means of antipest serum have given less favorable results, as shown in the following table:

Nasal inoculation with splenic pulp.		Result of disease.		Nasal inoculation with splenic pulp.		Result of disease.	
Quantity of serum injected.	Time of administration immediately after inoculation.	Cure.	Death.	Quantity of serum injected.	Time of administration immediately after inoculation.	Cure.	Death.
Cubic centimeters.	*Hours.*	*Days.*	*Death.*	*Cubic centimeters.*	*Hours.*	*Days.*	*Death.*
3	1½	6½	Do.	5	24	7½	Do.
3	4	9½	Do.	5	40	5	Do.
3	7	9½	Do.	5	....	4½	Do.
5	15	8½	Do.	Control.			

This picture is very instructive. It shows us that 3 c. c. of serum injected at the moment of inoculation may prevent the development of the pneumonia, but that even one-half hour later this same quantity only serves to prolong the disease. If we wish to obtain this result seven hours after the inoculation a much larger quantity of the serum is necessary, and finally there comes a time when, despite the injection of serum, the animals die in practically the same time as the controls.

It is then much easier to prevent than to cure plague-pneumonia (primary) with antipest serum, and this is easily explained when we remember what we have said above about secondary plague-pneumonia, that is that in the lung, the microbe finds extremely favorable conditions for its development, and that in this situation it is very inaccessible to the protective cells of the economy. We must also remark that among many animals dead, in spite of the administration of the serum, we find at the necropsy not only the plague bacillus, but many other foreign organisms; consequently these animals have succumbed to secondary infections, the soil for which has been prepared by the plague baccillus, primarily.

The picture of which we have just spoken leads us to certain general reflections. Thus we see that not only is it necessary to have a good serum, but that it is necessary to use it at the right time. This is above all important when we have to deal with a septicæmic disease like plague, where we must count on a rapid and enormous multiplication of the microbe. It is a well established fact at present that the serum does not act through its own power (the bacillus grows in the serum itself), but through the intermediary of certain cells of the organism whose activity is excited by it. Now the number of these cells is always limited, while that of the microbe which multiplies itself by certain fixed rules and by geometrical progression, increases in fantastic proportions, so that after a certain time all hope of intervention becomes illusory. In practice, account is not generally taken of this fact, and we often require more of serum than it is possible for the latter to give.

In plague, especially, it should be always a rule to pay attention to the form of the disease and the time which has elapsed since infection when we use the serum. Primary plague-pneumonia is a form of the disease which is difficult to cure with antipest serum; this is demonstrated by our experiments.

CONCLUSIONS.

An animal may contract the two forms of plague-pneumonia which are observed among mankind, viz, primary plague-pneumonia and secondary pneumonia.

2. Experimental primary plague-pneumonia is a lobular or confluent broncho-pneumonia, which ends generally in a septicæmia. It can be produced in all laboratory animals by depositing upon their nasal mucous membrane without excoriating it a little plague virus taken either from an agar culture, or, better still, from the spleen of a plague-stricken animal.

3. Plague-pneumonia is transmissible from animal to animal by contact. The secretions of the sick animal, especially the tears, nasal and bronchial mucus, placed or carried into the nose of a healthy animal, give it the disease.

4. A plague virus which does not kill by inoculation gives the pneumonia to an animal when it is introduced into its respiratory passages; after several successive passages through the nose the attenuated virus regains all its virulence.

5. Dried plague virus in albuminous materials is capable of inducing plague-pneumonia in an animal by nasal inoculation, after several weeks drying.

6. Secondary plague-pneumonia is developed in the guinea pig during the course of any plague infection, regardless of the avenue of entrance, as a consequence of a natural resistance acquired by the animal against the virus. In anatomical form it is a singular pneumonia leading to the formation of pseudo-tubercles on the surface of the lungs.

7. With antipest serum we can very often prevent the occurrence of plague-pneumonia (primary) but it is difficult to cure it when once it has commenced.

8. All the accessible mucous membranes of the body lend themselves more or less readily to penetration by plague virus. According to their degree of sensibility they may be classified as follows: Nasal mucous membrane, conjunctiva, mucous membrane of the mouth, of the intestine, and lastly that of the vagina.